The Role of Transgenic Mouse Models in Carcinogen Identification

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In this article, we examine existing data on the use of transgenic mouse models for identification of human carcinogens. We focus on the three most extensively studied of these mice, Trp53+/–, Tg/AC, and RasH2, and compare their performance with the traditional 2-year rodent bioassay. Data on 99 chemicals were evaluated. Using the International Agency for Research on Cancer/Report on Carcinogens determinations for the carcinogenicity of these chemicals to humans as the standard for comparison, we evaluated a variety of potential testing strategies ranging from individual transgenic models to combinations of these three models with each other and with traditional rodent assays. The individual transgenic models made the "correct" determinations (positive for carcinogens; negative for noncarcinogens) for 74–81% of the chemicals, with an increase to as much as 83% using combined strategies (e.g., Trp53+/– for genotoxic chemicals and RasH2 for all chemicals). For comparison, identical analysis of chemicals in this data set that were tested in the 2-year, two-species rodent bioassay yielded correct determinations for 69% of the chemicals. However, although the transgenic models had a high percentage of correct determinations, they did miss a number of known or probable human carcinogens, whereas the bioassay missed none of these chemicals. Therefore, we also evaluated mixed strategies using transgenic models and the rat bioassay. These strategies yielded approximately 85% correct determinations, missed no carcinogens, and cut the number of positive determinations for human noncarcinogens in half. Overall, the transgenic models performed well, but important issues of validation and standardization need further attention to permit their regulatory acceptance and use in human risk assessment. *Key words:* **carcinogens, hazard identification, mouse model, mutagenesis screening, transgenic models.** *Environ Health Perspect* **111:444–454 (2003). doi:10.1289/ehp.5778 available via** *http://dx.doi.org/* **[Online 30 October 2002]**

The National Toxicology Program (NTP) is responsible for evaluating the toxicity and carcinogenicity of environmental agents, developing and validating improved testing methods, and strengthening the science base of toxicology. A variety of end points are used to assess the systemic toxicity of environmental chemicals, but the mainstay of chemical carcinogenicity testing has been the 2-year rodent bioassay. This highly standardized method has been widely adopted throughout the world. However, like any other approach, the rodent bioassay has its strengths and weaknesses. In particular, the 2-year bioassay is expensive, both in resources and time required and in the numbers of animals needed. Thus, the advent of transgenic and gene knockout technology in the early 1980s and increasing knowledge of the mechanisms involved in carcinogenesis led a number of investigators to examine whether faster, less costly, and more predictive models might be developed. The National Institute of Environmental Health Sciences (NIEHS) has been actively involved in this effort for more than a decade, and several model systems using transgenic and knockout models have been investigated (Bucher 1998; Eastin et al. 1998; Tennant 1993; Tennant et al. 1995).

Transgenic models have a number of potential advantages for use in carcinogen identification programs. For example, because tumors arise more quickly in the genetically engineered models, the assays can be more rapid. For the studies reviewed here, the assay length was 24–26 weeks, significantly shorter than the standard 2-year rodent bioassay. Transgenic models may also provide the opportunity to reduce the number of animals used in testing. Shorter assays using fewer animals could also reduce the overall cost of testing programs. However, proprietary issues and the limited availability of some models may impact cost savings. Furthermore, with appropriate model selection, it may become possible to more accurately predict the human response, contributing directly to the ease and effectiveness of risk assessment and regulatory decisions. Finally, by virtue of the specific genetic modification(s) in transgenic models, it should be possible to gain additional insights into the mechanisms involved in tumor induction and development. Such insights would facilitate identification of important mechanisms of the tumor response and chemical features associated with carcinogenesis.

Although they have great promise, transgenic models also have actual or potential limitations for use in a carcinogen identification effort. For example, many current transgenic models (including those evaluated here) have mutations in only one pathway that may or may not be relevant to human cancer processes for a given chemical. In addition, the specific gene defect may influence tumor development and type, increasing the difficulty of modeling the human response. Likewise, the strain (genetic) background can influence tumor type, incidence, and location. Thus, short-term, gene-specific transgenic assays may lose biological information obtained in longer term bioassays (e.g., multiple target organ effects and/or interactions of time and age that are important in chemical carcinogenicity). These issues do not preclude the use of transgenic models, but they must certainly be considered in their development and selection and in interpretation of data obtained using transgenic models.

Given the potential and the limitations of the transgenic models, the goals of the current assessment were to *a*) review progress in this field of research, *b*) determine if the models reviewed show sufficient merit for use in a carcinogen identification program, and *c*) identify research needs and knowledge gaps that should be addressed to increase the effectiveness of transgenic models.

Review of Research Progress

Many transgenic models are available for various investigational uses, but three transgenic models have been most widely used for carcinogen identification: Trp53+/–, Tg.AC, and RasH2. We selected these three models for this assessment because they have the extensive data set needed for this analysis. Their selection does not indicate that they are deemed superior *a priori* to other transgenic models.

Extensive recent reviews of these three models have been published, and only their main features are briefly reviewed here. They were developed based on dysregulation of either the Trp53 tumor-suppressor gene or the *ras*-proto-oncogene, both of which are critical to cancer development and which represent the two main classes of human cancer genes. The p53 protein suppresses cancer in humans and rodents and is mutated or dysfunctional in more than 50% of all cancers (Donehower et al. 1992; Hollstein et al. 1991; Weinberg 1991a). As a transcription factor, p53 regulates the activity of a variety of genes involved in cell cycle arrest, apoptosis, anti-angiogenesis, differentiation, DNA repair, and genomic stability

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